Institute Report No. 417

Dermal Sensitization Potential of JA-2 Solid Propellant in Guinea Pigs

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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

November 1989

Toxicology Series: 182

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Dermal Sensitization Potential of JA-2 Solid Propellant in Guinea Pigs (Toxicology Series 182)--Lewis *et al.*

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This research was conducted in compliance with the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23, as prepared by the Institute of Laboratory Animal Resources, National Research Council.

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Donald G. Corby 29 Nov 89
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COL, MC
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JA-2 Solid Propellant was evaluated for its potential to produce dermal sensitization in male guinea pigs. The Buehler test, which utilizes repeated closed patch inductions with the test compound, was used for this evaluation. No evidence that JA-2 Solid Propellant induced sensitization was obtained in the study.

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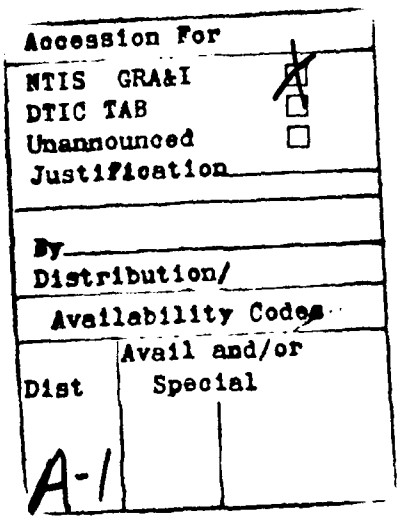
ABSTRACT

JA-2 Solid Propellant was evaluated for its potential to produce dermal sensitization in male guinea pigs. The Buehler test, which utilizes repeated closed patch inductions with the test compound, was used for this evaluation. No evidence that JA-2 Solid Propellant induced sensitization was obtained in the study.

Key Words: Dermal Sensitization, Mammalian Toxicology, JA-2 Solid Propellant, Buehler Test, Nitroglycerin, Diethyleneglycol Dinitrate, Guinea Pig, Propellant



PREFACE

TYPE REPORT: Dermal Sensitization GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NO.: 85020

STUDY DIRECTOR: Don W. Korte, Jr., PhD, LTC, MSC
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PRINCIPAL INVESTIGATOR: Carolyn M. Lewis, MS
Diplomate, American Board of Toxicology

CO-INVESTIGATOR: Larry D. Brown, DVM, LTC, VC, Diplomate,
American College of Veterinary Preventive Medicine,
American Board of Toxicology.

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: JA-2 Solid Propellant

INCLUSIVE STUDY DATES: 4 April 1986 - 19 May 1986.

OBJECTIVE:

The objective of the study was to evaluate the dermal sensitization potential of JA-2 Solid Propellant in guinea pigs.

ACKNOWLEDGMENTS

SSG James D. Justus, SP4 Scott L. Schwebe, SP4 Theresa L. Polk, PFC Joel B. Seewald, Richard A. Spieler, and Obie Goodrich assisted in the dosing, scoring, and care of the animals. Colleen S. Kamiyama and Dorothy Davis provided administrative and clerical support during the performance of the study and preparation of the report.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85020 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr 27 Nov 89

DON W. KORTE, JR. PhD / DATE
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Study Director

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Analytical Chemist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

29 November 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85Ø2Ø

1. This is to certify that the protocol for LAIR GLP Study 85Ø2Ø was reviewed on 1Ø May 1985.

2. The institute report entitled "Dermal Sensitization Potential of JA-2 Solid Propellant in Guinea Pigs," Toxicology Series 182, was audited on 15 November 1989.

Walter G. Bell
WALTER G. BELL
SFC, USA
Quality Assurance Officer

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INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in new propellant formulations. However, considerable gaps in the toxicology data of the compounds were identified during a review of their health effects (1) conducted for the US Army Biomedical Research and Development Laboratory (USABRDL). Consequently, USABRDL has tasked the Division of Toxicology, Letterman Army Institute of Research (LAIR), to conduct an initial health effects evaluation of the proposed replacement nitrate esters. This initial evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP, includes the Ames mutagenicity assay, acute oral toxicity studies in rats and mice, acute dermal toxicity study in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the dermal sensitization potential of JA-2 Solid Propellant in guinea pigs.

MATERIALS

Test Substance

Chemical Name: JA-2 Solid Propellant

LAIR Code Number: TP56

Description: Solid black cylinders (stick configuration)

Lot Number: RAD83K001S153

JA-2 Solid Propellant was received in the stick configuration and ground into a fine powder for this study. Other test substance information is presented in Appendix A.

Vehicle

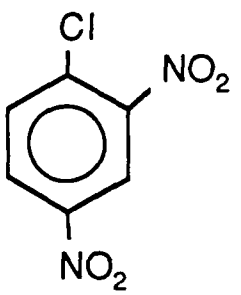
Isotonic saline (Viaflex®, Sodium Chloride Injection, USP; Travenol Laboratories, Inc., Deerfield, IL, lot # 3C979X6) was used as the vehicle for the test compound and as a component of the positive control vehicle.

Positive Control

Chemical Name: Dinitrochlorobenzene (DNCB)

Chemical Abstracts Service Registry No.: 97-00-7

Chemical Structure:



Molecular Formula: $C_6H_3N_2O_4Cl$

Other positive control substance information is presented in Appendix A.

Vehicle for Positive Control

A 0.1% solution of DNCB was prepared fresh for each induction dosing and the challenge dosing. The vehicle for DNCB was a propylene glycol (3%) and isotonic saline (97%) mixture. Propylene glycol (lot number 36485) was obtained from Certified Laboratories, Inc. (Philadelphia, PA).

Animal Data

Sixty-six male albino guinea pigs, Hartley strain (Charles River Breeding Laboratories, Wilmington, MA), were received for this study. They were identified individually with ear tags numbered 86E00197 to 86E00262, inclusive. Two animals (86E00217, 86E00243) were selected for quality control necropsy evaluation on receipt. Four animals were selected for a pilot study to determine a non-irritating dose level. Animal weights on the day of receipt ranged from 177 to 232 g. Additional animal data appear in Appendix B.

Husbandry

Guinea pigs assigned to this study were caged individually in stainless steel, wire mesh cages in racks equipped with automatically flushing dump tanks. The diet, fed *ad libitum*, consisted of Certified Purina Guinea Pig Chow® Diet 5026 (lot nos. JAN03861A, FEB13862A, MAR18862A, and MAR24862A; Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by continuous drip from a central line. Temperature within the animal room was maintained in the range from 20.0 to 24.4°C. Relative humidity was maintained in the range of 35% to 50%. Spikes in room temperature and relative humidity occurred occasionally during room cleaning. The photoperiod was 12 hours of light per day.

METHODS

This study was conducted in accordance with LAIR SOP-OP-STX-82, "Buehler Dermal Sensitization Test," (2) and EPA guidelines (3).

Group Assignment/Acclimation

The guinea pigs were quarantined for 12 days before administration of the first induction dose. During the quarantine period, they were checked daily for signs of illness and weighed once a week. Fifteen animals were assigned to each of three groups by a stratified randomization technique based on their body weights.

Dose Levels

A pilot study using 100%, 10%, 1%, and 0.1% concentrations of JA-2 in isotonic saline, was conducted to determine the maximal nonirritating concentration. No irritation was evident from the test compound at any concentration. Therefore, 100% JA-2 moistened with 0.5 ml of isotonic saline was employed in this study.

Four animal groups comprise the basis for this report. Dermal sensitization potential was evaluated in a test group receiving three weekly induction doses of 100% JA-2 and, after a two-week delay, a challenge dose at the same concentration. Dinitrochlorobenzene, a known potent sensitizing agent (4), was applied to another group as a positive control The vehicle, isotonic saline, was applied to a third group. A negative control group received 100% JA-2 only on the day of challenge dosing.

Compound Preparation

The test compound was prepared by mixing 0.5 g of JA-2 with 0.5 ml of 0.9% saline to make a paste. The dinitrochlorobenzene (DNCB) dosing solution was prepared by first adding 15 mg DNCB to 0.5 ml of propylene glycol and heating until it dissolved (approximately 40°C). To this, 14.5 ml of isotonic sodium chloride solution were added, to give a final concentration of 0.1% (w/v). This solution was heated to 65°C and vortexed before application to keep the DNCB in solution. DNCB solutions were prepared fresh for each application day.

Test Procedures

The closed patch dermal sensitization test procedures utilized in this study were developed by Buehler and Griffith (5-7) to mimic the repeated-insult patch test for humans. Test compounds were applied for six hours under a closed patch once a week for three weeks during the induction phase. The same application site was used for each induction dose. To distinguish between reactions from repeated insult and sensitization, duplicate patches of the challenge dose were applied, one on the old site and one on a new site.

To distinguish between reactions from primary irritation and sensitization, a negative control group was added which received only the challenge dose.

During the induction phase, the test and positive control groups were dosed with 0.5 ml of the appropriate compound/suspension applied topically under a 2.5-cm^2 gauze patch. This procedure was performed for three consecutive weeks (16, 23, and 30 Apr). Twenty-four hours before each dosing, a 7.6-cm^2 area on the left flank of the animal was clipped with electric clippers (Oster® Model A5, size 40 blade, Sunbeam Corp., Milwaukee, WI) and then shaved with an electric razor (Norelco® Speed Razor Model HP1134/S, North American Phillips Corp., Stamford, CT). The patch was taped with Blenderm® hypoallergenic surgical tape (3M Corp., St. Paul, MN) to the same site each time, and the animal was wrapped several times with Vet Wrap® (3M Corp., St. Paul, MN). The patch was left in place for six hours. When the wrap and patch were removed, the area under the patch was gently wiped of any excess compound using a saline-moistened gauze and the site was marked for scoring.

Animals were challenged two weeks (14 May) following the third induction dose. Test group and positive control group animals received two 0.5-ml doses each of JA-2 or DNCB, respectively, one applied to the old site on the left flank and the other to a new site on the right flank. Negative control and vehicle control animals received only a single 0.5-ml dose of JA-2 or isotonic saline, respectively, applied to the left flank. Procedures for clipping, shaving, and wrapping and the exposure period remained the same.

In Buehler's procedure, skin reactions are scored 24 and 48 hours after the challenge dose only. In the present study, skin reactions were scored 24, 48, and 72 hours after each induction dose as well as 24, 48, and 72 hours after the challenge dose. Skin reactions were assigned scores according to Buehler's grading system: 0 (no reaction), 1 (slight erythema), 2 (moderate erythema), and 3 (marked erythema). Results are expressed in terms of both incidence (the number of animals showing responses of 1 or greater at either 24, 48, or 72 hours) and severity (the sum of the test scores

divided by the number of animals tested). Results from the left flank are compared with right flank and with the negative control group.

Some modifications of Buehler's procedures were made. Instead of placing animals in restraint during the 6-hour exposure period, the animals were wrapped several times with an elasticized tape to hold the patch in place. Consequently, the animals were able to move about freely in their cages during the exposure period. Buehler and Griffith (7) also recommended depilating the day before the challenge dose. For consistency with induction procedures, this step was replaced by clipping the animals.

The animals were observed daily for clinical signs and weight gain was monitored during the study. At the conclusion of the study, a necropsy was performed on each animal. A historical listing of study events appears in Appendix C.

Changes/Deviations

This study was conducted in accordance with the protocol and applicable amendments.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Experimental

Table 1 summarizes the incidence of reactions 24, 48, and 72 hours after each dose. No reaction was observed in response to JA-2 after any of the induction doses or the challenge dose. This lack of response is reflected in Table 2 which depicts the severity of skin reactions. Response severity for each group is calculated by summing the scores of responding animals and

dividing by the total number of animals within that group. For JA-2 no responses were obtained; therefore, severity scores were zero at all times.

Positive Control

Dinitrochlorobenzene produced a marked response at all time points after the first induction dose (Table 1). Between 73% and 100% of the DNCB-treated animals exhibited a response 24 hours following the second or third induction and challenge doses. These reactions persisted, yielding scorable effects in 100% of the animals at 48 hours after dosing and 60-100% of the animals at 72 hours after dosing. Severity scores for these responses to DNCB ranged from 0.0 to 1.67 at the 24-hour scoring period (Table 2). The highest score, 1.67, was observed in response to the challenge dose on the left flank. By 48 hours the reactions had increased slightly with a severity range between 0.0 and 1.80. At 72 hours the reactions had subsided to a range of 0.0 to 1.20.

Negative and Vehicle Controls

No response was observed in the negative control (challenge dose of JA-2) group or in the vehicle control group. Individual 24-hour, 48-hour, and 72-hour dermal scores for all animals appear, by group, in Appendix D.

Clinical Signs

All animals were healthy and gained weight during the study. Individual body weight data are presented in Appendix E.

Pathology Findings

A necropsy was performed on all study animals. Minimal white multifocal lesions were observed in the livers of several animals in the experimental, positive control, and negative control groups. These lesions were considered incidental and of minimal clinical or pathological significance. The complete pathology report is presented in Appendix F.

TABLE 1: Incidence of Skin Reactions

Test Group	Induction			Challenge	
	First	Second	Third	Left	Right
24 Hours					
Vehicle Control	0/15	0/15	0/15	0/15	—
DNCB	0/15	13/15	11/15	15/15	9/15
JA-2	0/15	0/15	0/15	0/15	0/15
Negative Control*	—	—	—	0/15	—
48 Hours					
Vehicle Control	0/15	0/15	0/15	0/15	—
DNCB	0/15	15/15	15/15	15/15	15/15
JA-2	0/15	0/15	0/15	0/15	0/15
Negative Control*	—	—	—	0/15	—
72 Hours					
Vehicle Control	0/15	0/15	0/15	0/15	—
DNCB	0/15	13/15	10/15	8/15	9/15
JA-2	0/15	0/15	0/15	0/15	0/15
Negative Control*	—	—	—	0/15	—

*The Negative Control Group received only a challenge dose of the test compound.

TABLE 2: Severity of Skin Reactions

Test Group	Induction			Challenge	
	First	Second	Third	Left	Right
			24 Hours		
Vehicle Control	0.0	0.0	0.0	0.0	—
DNCB	0.07	1.13	0.87	1.67	0.60
JA-2	0.0	0.0	0.0	0.0	0.0
Negative Control*	—	—	—	0.0	—
			48 Hours		
Vehicle Control	0.0	0.0	0.0	0.0	—
DNCB	0.0	1.20	1.53	1.86	1.07
JA-2	0.0	0.0	0.0	0.0	0.0
Negative Control*	—	—	—	0.0	—
			72 Hours		
Vehicle Control	0.0	0.0	0.0	0.0	—
DNCB	0.0	0.93	1.20	1.00	0.60
JA-2	0.0	0.0	0.0	0.0	0.0
Negative Control*	—	—	—	0.0	—

*The Negative Control Group received only a challenge dose of the test compound.

DISCUSSION

Dermal Irritation and Sensitization

Most skin reactions occurring from contact with chemicals can be classified as either irritation or sensitization. Both reactions present as inflammation of the skin; the difference between irritation and sensitization is the mechanism responsible for this inflammation. Primary irritation is direct inflammation in response to injury to the skin produced by the eliciting chemical. Irritation is a locally mediated response ranging from mild reversible inflammation to severe ulceration progressing to necrosis. Sensitization is manifested as indirect inflammation mediated by components of the immune system in response to activation by the eliciting chemical (8). Dermal sensitization is usually a delayed hypersensitivity or cellular immunologic reaction. Although both types of reactions can appear grossly similar in experimental animals and may even be produced by the same agent, it is possible to distinguish between them. Irritation is an immediate response and can be produced upon first contact with the chemical, whereas sensitization requires at least one innocuous "conditioning" exposure before a reaction can be elicited.

Irritative responses usually require a relatively high concentration or dose of the offending chemical, whereas sensitization reactions may occur in response to minute quantities. Essentially all individuals in a population will express an irritative response to a reactive chemical, provided the dose is high enough, whereas only a fraction of the population normally becomes sensitized to the same chemical. A fully developed response can be produced by first contact with an irritant, but initial contact with a sensitizer produces no reaction (a conditioning exposure is necessary). Unless there is accumulation of damage, subsequent exposures to an irritant produce inflammation of essentially similar intensity/severity, whereas the reaction to a sensitizer often increases over 2 to 4 exposures after the initial contact. An irritant produces inflammation of rapid onset with short duration, whereas a sensitization reaction is somewhat delayed and prolonged. The inflammatory response to

an irritant may spread beyond the area of contact, whereas sensitization reactions are usually circumscribed.

The features of irritation and sensitization have been used to establish guidelines for differentiation between the two (5-8). In evaluating a dermal sensitization study it is recommended that the results from a challenge dose in the experimental group (sensitization) be compared with those for the negative control group (irritation) in accordance with the following criteria:

Irritative Responses:

- occur in a large proportion of test animals.
- develop in response to the first or second exposure.
- usually fade within 24 to 48 hours, unless damage is severe.
- may be stronger at challenge to a previously unexposed area of skin (contralateral flank).

Sensitization Reactions:

- occur in only a few animals, unless the compound is a potent sensitizer.
- are absent after the initial (conditioning) exposure, but appear in response to subsequent exposures.
- develop slowly with the intensity/severity of inflammation often greater at 72 to 96 hours than at 24 to 48 hours.
- increase in intensity/severity from one exposure to the next (at sites previously exposed or unexposed).

Dermal irritancy potential is evaluated by the method of Draize *et al.* (9) in which the chemical is applied once, at high concentration, and the resulting acute inflammatory reaction is graded. Evaluation of sensitizing potential is accomplished by repeated application, at lower nonirritating concentrations, over a few weeks. There is then a latent period, usually two weeks, to allow the immune system to elaborate and increase its specific response to the chemical. A challenge dose is then given, and the resulting inflammatory response is graded. Analysis of the incidence, severity, and timing of the response to the challenge dose estimates the sensitizing potential of the study compound.

JA-2 Solid Propellant

JA-2 Solid Propellant was evaluated for its ability to elicit a delayed hypersensitivity or cellular immunologic reaction via contact with the skin. JA-2 produced no response indicative of the potential to elicit dermal sensitization when evaluated according to the method of Buehler and Griffith (5-7).

Sensitization produced by JA-2 would have been detected by this study. A hypersensitivity-type response was reliably elicited by DNCB in the present group of animals. This response to DNCB was characteristic of that observed previously at the Letterman Army Institute of Research (10). Although DNCB is capable of producing primary irritation, the characteristics of the responses observed in this study are indicative of a reaction due to sensitization. The concentration of DNCB used for induction and challenge is too low to produce primary irritation. Also, the response to DNCB was observed primarily after two or more exposures.

Because the guinea pig exhibits a somewhat lower sensitizing responsiveness than does man, this result does not guarantee that JA-2 will not sensitize humans. However, it does indicate that JA-2 is unlikely to sensitize humans and its potential is low enough to permit its evaluation in man.

CONCLUSION

JA-2 Solid Propellant possesses minimal sensitizing potential, as it did not induce a dermal sensitization reaction under conditions of this study.

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Appendix A: CHEMICAL DATA

Test substance: JA-2 Solid Propellant

LAIR Code Number: TP56

Physical State: Solid black cylinders (stick configuration)

Preparation of test substance for dosing: The cylinders of JA-2 were ground to a fine powder under liquid nitrogen using a Spex freezer mill. The powder was then sieved through an 80-mesh screen.

Chemical Analysis:

DEGDN was the only major component of JA-2 that could be easily analyzed.[1] To determine the percent DEGDN in the JA-2 propellant, samples of JA-2 powder were added to individual 100 ml volumetric flasks. After dilution to volume with 95% ethanol, a second 1:100 dilution was performed. These solutions were analyzed by HPLC. Standards consisted of solutions of DEGDN in ethanol ranging in concentration from 164.5 to 670.5 µg/ml. Analysis of DEGDN by HPLC was performed under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm, Brownlee Labs, Inc., Santa Clara, CA); solvent system, 40% water - 60% acetonitrile); flow rate, 0.9 ml/min; wavelength monitored, 210 nm.[2] Under these conditions, DEGDN eluted with a retention time of approximately 5.4 min.

The results from the analysis of standards and JA-2 powder samples are presented in Tables 1 and 2.

Table 1. Analysis of standards

Concentration of Standard (µg/ml)	Peak Area* ($\times 10^{-7}$)
164.5	0.94
191.0	1.09
275.5	1.60
299.4	1.74
362.0	2.08
399.6	2.31
444.4	2.52
539.8	3.07
585.0	3.32
670.5	3.79

*Average of 2 determinations

Equation for line by linear regression analysis:

$Y = 5.62 \times 10^4 X + 3.51 \times 10^5$, $r^2 = 0.9999$

Appendix A (cont.): CHEMICAL DATA

Table 2. Analysis of JA-2 Powder

Weight of JA-2 Analyzed (mg)	Dilution Factor	Peak Area (x 10-7)	Conc. of DEGDN in JA-2 (weight %)*
104.8	100	1.56	25.9
101.6	100	1.57	26.9
109.7	100	1.69	26.8

*Calculated using the equation for the standard curve as follows:
= {[Peak Area - 3.51 x 10^5]/5.62 x 10^4} ÷ wgt JA-2 (mg) x 10.

The average value for the concentration of DEGDN in JA-2 was 27% and this agrees closely with the value of 24.82 ± 1.50 % reported in the data sheet provided by the source.

Stability: The aqueous stability of the DEGDN component of JA-2 propellant was determined.[3] The amount of DEGDN in JA-2 suspensions was determined immediately after preparation of a suspension and again 24 hours later. The study was conducted as follows: A suspension of JA-2 in 1% gum tragacanth (200 mg/ml) was prepared. Three 1 ml aliquots were removed from the suspension immediately after preparation and again 24 hours later. The 1 ml samples were transferred to individual 100 ml volumetric flasks. After diluting to volume with ethanol, the solutions were analyzed by HPLC as described above. The average of the peak area values was 2.92 ± 0.12 for the 0 time samples and 2.95 ± 0.11 for the 24-hour samples. These results indicate that there was no decomposition of DEGDN in 1% gum tragacanth for a period of 24 hours.

Source: Radford Army Ammunition Plant, Radford, VA
(prime contractor: Hercules, Inc., Wilmington, DE)

Lot no.: RAD83K001S153

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[1] Wheeler CR. Toxicity testing of propellants. Laboratory Notebook #85-12-023, p. 51-61. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[2] Wheeler CW. Nitrocellulose-nitroguanidine projects. Laboratory Notebook #84-05-010.3, p. 58. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3] Wheeler CR. Toxicity testing of propellants. Laboratory Notebook #85-12-023, p. 27, 35, 41. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

CHEMICAL ANALYSIS FOR JA-2
(Information from the Manufacturer's Data Sheet)

Ingredient	Finished Propellant Percentage
Nitrocellulose (13.8% ±0.05% Nitrogen) (6-12 seconds viscosity)	58.5 ±2.00
Nitroglycerin	15.88 ±1.00
Diethyleneglycol dinitrate (DEGDN)	24.82 ±1.50
Akardit II	0.70 ±0.20
Magnesium Oxide	0.04 Max
Graphite	0.04 Max
Total	100.00%*

*Data provided as listed; total actually equals 99.98%.

Appendix B: ANIMAL DATA

Species: *Cavia porcellus*

Strain: Hartley, albino

Source: Charles River Breeding Laboratories
Wilmington, MA

Sex: Male

Date of Birth: 17 March 1986

Method of randomization: Weight bias, stratified animal allocation

Animals in each group: 15 male animals

Condition of animals at start of study: Normal

Identification procedures: Ear tag.

Pretest conditioning: Quarantine/acclimation 4-15 April 1986

Justification: The laboratory guinea pig has proven to be a sensitive and reliable model for detection of delayed hypersensitivity from dermal contact.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

Date	Event
4 Apr 86	Animals arrived at LAIR. Animals were examined, weighed, placed in cages, and fed. Animals were assigned ear tags. Two animals were submitted for necropsy quality control.
5-16 Apr 86	Animals were observed daily.
7,15,22,29 Apr, 6,13,19 May 86	Animals were weighed.
7-10 Apr 86	Four pilot animals were clipped and shaved, and dosed with the test compound at 100%, 10%, 1%, and 0.1% concentrations on four sites/animal. Skin reaction was scored at 24 and 48 hours.
15 Apr 86	Animals were randomized into groups.
15,22,29 Apr 86	All animals, except negative control group, were clipped and shaved.
16,23,30 Apr 86	All animals, except negative control group, were given induction dose.
17,24 Apr, 1 May 86	All animals, except negative control group, were scored for 24-hour skin reaction.
18,25 Apr, 2 May 86	All animals, except negative control group, were scored for 48-hour skin reaction.
19,26 Apr, 3 May 86	All animals, except negative control group, were scored for 72-hour skin reaction.
13 May 86	All animals were clipped and shaved.
14 May 86	All animals were given a challenge dose.
15 May 86	All animals were scored for 24-hour skin reaction.

Appendix C (cont.): HISTORICAL LISTING OF STUDY EVENTS

Date	Event
16 May 86	All animals were scored for 48-hour skin reaction.
17 May 86	All animals were scored for 72-hour skin reaction.
19 May 86	All animals were delivered to the Necropsy Suite for gross necropsy.

Appendix D: INDIVIDUAL ANIMAL SCORES

GROUP: ONE

COMPOUND: Vehicle Control

ANIMAL NUMBER	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE DOSE					
										RIGHT FLANK			LEFT FLANK		
	24 H	48 H	72 H	24 H	48 H	72H	24 H	48 H	72H	24 H	48 H	72 H	24 H	48 H	72 H
86E0203	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0207	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0218	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0224	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0228	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0235	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0240	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0244	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0249	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0250	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0251	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0252	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0253	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0254	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0280	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0

Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

GROUP: TWO

COMPOUND: DNCB

ANIMAL NUMBER	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE DOSE					
										RIGHT FLANK			LEFT FLANK		
	24 H	48 H	72 H	24 H	48 H	72H	24 H	48 H	72H	24 H	48 H	72 H	24 H	48 H	72 H
86E0198	0	0	0	0	1	0	0	1	0	0	1	0	1	1	0
86E0202	0	0	0	1	1	1	1	2	2	1	1	1	2	2	1
86E0209	0	0	0	1	1	1	1	2	1	1	1	0	2	2	0
86E0210	0	0	0	2	2	1	1	1	0	1	1	0	1	1	0
86E0214	0	0	0	1	1	1	1	1	0	0	1	1	2	1	0
86E0215	0	0	0	1	1	1	1	1	1	0	1	1	1	2	0
86E0219	0	0	0	0	1	1	0	1	0	0	1	0	2	2	0
86E0220	0	0	0	2	1	1	1	1	2	0	1	1	1	2	2
86EC231	0	0	0	1	1	1	0	1	0	1	1	1	2	2	0
86E0233	0	0	0	1	1	0	2	3	3	1	1	1	2	3	2
86E0234	0	0	0	2	2	2	1	2	2	1	1	1	1	2	2
86E0242	0	0	0	1	1	1	0	1	1	0	1	0	1	1	1
86E0246	0	0	0	1	1	1	1	2	2	1	1	1	2	2	2
86E0247	0	0	0	1	1	1	1	1	1	1	1	0	2	2	2
86E0262	0	0	0	2	2	1	2	3	3	1	2	1	3	3	3

Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

GROUP: THREE

COMPOUND: JA-2

ANIMAL NUMBER	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE DOSE					
										RIGHT FLANK			LEFT FLANK		
	24 H	48 H	72 H	24 H	48 H	72H	24 H	48 H	72H	24 H	48 H	72 H	24 H	48 H	72 H
86E0197	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0199	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0205	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0206	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0208	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0212	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0222	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0223	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0227	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0236	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0237	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0238	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0255	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0261	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

GROUP: FOUR

COMPOUND: NEGATIVE CONTROL

ANIMAL NUMBER	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE DOSE					
										RIGHT FLANK			LEFT FLANK		
	24 H	48 H	72 H	24 H	48 H	72H	24 H	48 H	72H	24 H	48 H	72 H	24 H	48 H	72 H
86E0201	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0204	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0211	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0213	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0216	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0221	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0225	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0226	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0229	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0232	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0239	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0245	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0248	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0256	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0259	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0

Appendix E: INDIVIDUAL BODY WEIGHTS (grams)

Vehicle Control

Animal Number	DAY OF STUDY							
	Q*0	Q3	Q11	6	13	20	27	33
86E00203	208	243	288	318	351	388	418	432
86E00207	204	232	291	328	375	401	442	468
86E00218	198	234	282	319	363	379	404	424
86E00224	229	259	316	343	379	415	447	462
86E00228	200	203	267	312	343	362	416	424
86E00235	205	253	326	388	450	511	566	590
86E00240	209	244	311	357	415	468	516	538
86E00244	231	257	307	334	367	389	436	441
86E00249	199	229	284	319	358	385	431	443
86E00250	190	227	274	326	372	413	469	492
86E00251	203	235	299	350	397	430	476	498
86E00252	232	270	313	321	354	383	411	428
86E00253	177	209	236	262	288	310	324	339
86E00254	203	246	304	340	384	413	456	480
86E00260	210	268	342	395	461	513	581	600
MEAN	207	241	296	334	377	411	453	471
Standard Deviation	15	19	26	32	42	54	65	67
Standard Error	4	5	7	8	11	14	17	17

* Q represents quarantine period.

Appendix E (cont.): INDIVIDUAL BODY WEIGHTS (grams)

DNCB

Animal Number	DAY OF STUDY							
	Q*0	Q3	Q11	6	13	20	27	30
86E00198	191	224	268	307	354	397	434	450
86E00202	196	226	274	321	343	397	429	447
86E00209	197	236	310	343	406	445	500	527
86E00210	211	250	315	286	357	428	474	501
86E00214	205	237	296	345	394	426	483	479
86E00215	204	230	297	339	372	415	461	461
86E00219	196	222	280	303	341	375	413	432
86E00220	199	243	305	349	386	423	434	466
86E00231	206	242	316	359	413	468	525	547
86E00233	226	268	332	375	412	446	478	507
86E00234	204	241	284	326	360	403	454	470
86E00242	216	257	317	356	379	397	435	442
86E00246	194	204	262	305	331	377	434	436
86E00247	211	234	290	316	338	397	445	469
86E00262	194	234	306	347	397	438	479	502
MEAN	203	236	397	332	372	415	458	475
Standard Deviation	10	15	20	25	28	27	31	34
Standard Error	2	4	5	6	7	7	8	9

* Q represents quarantine period.

Appendix E (cont.): INDIVIDUAL BODY WEIGHTS (grams)

JA-2 SOLID PROPELLANT

Animal Number	DAY OF STUDY							
	Q*0	Q3	Q11	6	13	20	27	33
86E00197	222	256	312	348	408	450	495	524
86E00199	191	221	284	331	376	420	470	487
86E00200	205	228	296	329	347	376	416	426
86E00205	194	230	296	337	395	413	458	452
86E00206	218	250	317	362	391	432	472	487
86E00208	19[illegible]	224	264	294	315	355	390	400
86E00212	1[illegible]5	233	302	363	403	441	488	516
86E00222	201	209	259	283	312	351	438	404
86E00223	203	237	287	312	367	395	387	466
86E00227	192	219	268	299	342	388	435	449
86E00236	215	250	311	356	399	430	480	492
86E00237	214	257	323	376	426	483	540	567
86E00238	224	261	326	365	399	431	497	514
86E00255	184	229	283	323	357	388	427	434
86E00261	228	259	308	338	377	417	467	492
MEAN	205	238	296	334	374	411	457	474
Standard Deviation	14	17	21	28	34	36	42	47
Standard Error	4	4	5	7	9	9	11	12

* Q represents quarantine period.

Appendix E (cont.): INDIVIDUAL BODY WEIGHTS (grams)

Negative Control

Animal Number	DAY OF STUDY							
	0*0	03	011	6	13	20	27	33
86E00201	213	237	290	327	368	404	438	457
86E00204	237	274	326	381	425	477	538	554
86E00211	214	255	314	357	392	440	494	529
86E00213	192	220	263	309	343	371	414	424
86E00216	224	260	326	359	402	434	462	475
86E00221	206	222	281	334	385	433	482	495
86E00225	197	227	292	343	387	431	482	495
86E00226	208	234	296	344	400	447	496	524
86E00229	188	210	271	323	377	411	442	479
86E00232	191	233	301	356	407	455	496	496
86E00239	186	217	284	328	374	416	460	462
86E00245	194	216	254	280	306	340	366	370
86E00248	211	247	307	342	389	436	474	493
86E00256	213	244	310	371	413	447	498	510
86E00259	207	260	316	376	428	474	538	548
MEAN	205	237	295	342	386	428	472	487
Standard Deviation	14	19	22	27	31	36	45	47
Standard Error	4	5	6	7	8	9	12	12

* Q represents quarantine period.

Appendix F: PATHOLOGY REPORT

GLP Study #85020

Principle Investigator: Ms. Carolyn Lewis APC# LLE0

I. INTRODUCTION

Study: Dermal Sensitization of JA2
Animal: Guinea Pig
Reference: SOP-OP-STX-82

II. SUMMARY OF PROCEDURES

Euthanasia: Sodium Pentobarbital.
Fixative: 10% buffered formalin.
Histopathology: None.
Clinical Lab: None.

III. GROSS FINDINGS

DOSE GROUP 1 - VEHICLE CONTROL
(All live animals)

LAIR ACC#	ANIMAL ID#	OBSERVATION
39633	86E00203	Not remarkable (NR)
39636	86E00207	NR
39646	86E00218	NR
39652	86E00224	NR
39656	86E00228	NR
39662	86E00235	NR
39667	86E00240	NR
39669	86E00244	NR
39674	86E00249	NR
39675	86E00250	NR
39676	86E00251	NR
39677	86E00252	NR
39678	86E00253	NR
39679	86E00254	NR
39683	86E00260	NR

Comment: Gross lesions were not observed.

DOSE GROUP 2 - POSITIVE CONTROL
(All live animals)

LAIR ACC#	ANIMAL ID#	OBSERVATION
39627	86E00198	NR
39632	86E00202	NR
39638	86E00209	NR
39639	86E00210	NR
39643	86E00214	NR
39644	86E00215	Focal, white focus, minimal, liver

Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 85020

DOSE GROUP 2 (Continued)

LAIR ACC#	ANIMAL ID#	OBSERVATIONS
39647	86E00219	Multifocal white foci, minimal, liver
39648	86E00220	Multifocal white foci, minimal, liver
39658	86E00231	Multifocal white foci, minimal, liver
39660	86E00233	NR
39661	86E00234	NR
39668	86E00242	Multifocal white foci, minimal, liver
39671	86E00246	NR
39672	86E00247	NR
39685	86E00262	NR

Comment: The lesions noted in five animals in this group were incidental and considered to be of minimal clinical or pathological significance.

DOSE GROUP 3 - EXPERIMENTAL
(All live animals)

LAIR ACC#	ANIMAL ID#	OBSERVATION
39626	86E00197	Multifocal white foci, minimal, liver
39628	86E00199	Multifocal white foci, minimal, liver
39629	86E00200	NR
39634	86E00205	NR
39635	86E00206	NR
39637	86E00208	NR
39641	86E00212	NR
39650	86E00222	NR
39651	86E00223	NR
39655	86E00227	NR
39663	86E00236	NR
39664	86E00237	NR
39665	86E00238	NR
39680	86E00255	NR
39684	86E00261	Multifocal white foci, minimal, liver

Comment: The lesions noted in three animals in this group were incidental and considered to be of minimal clinical or pathological significance.

Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 85020

DOSE GROUP 4 - NEGATIVE CONTROL
(All live animals)

LAIR ACC#	ANIMAL ID#	OBSERVATION
39630	86E00201	NR
39631	86E00204	Multifocal white foci, minimal, liver
39640	86E00211	NR
39642	86E00213	NR
39645	86E00216	NR
39649	86E00221	Multifocal white foci, minimal, liver
39653	86E00225	NR
39654	86E00226	Multifocal white foci, minimal, liver
39657	86E00229	NR
39659	86E00232	NR
39666	86E00239	Multifocal white foci, minimal, liver
39670	86E00245	Multifocal white foci, minimal, liver
39673	86E00248	NR
39681	86E00256	NR
39682	86E00259	Multifocal white foci, minimal, liver

Comment: The lesions noted in the six animals in this group were incidental and considered to be of minimal clinical or pathological significance.

HARRY L. WALKER, DVM
CPT, VC
Comparative Pathology Branch

G. TRACY MAKOVEC, DVM
MAJ, VC
Diplomate, ACVP
Comparative Pathology Branch

21 May 1986

Distribution List

Commander
US Army Biomedical Research and Development Laboratory (12)
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center (DTIC) (2)
ATTN: DTIC-DLA
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and Development Command (2)
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEHA Regional Division, North
Fort George G. Meade, MD 20755

Chief
USAEHA Regional Division, South
Bldg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commander US Army Materiel Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

Commander
US Army Environmental Hygiene Agency
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ATTN: DASG-PSP-E
Falls Church, VA 22041-3258

HQDA
ATTN: DAEN-RDM
20 Massachusetts, NW
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CDR, US Army Toxic and Hazardous Material Agency
ATTN: DRXTH/ES
Aberdeen Proving Ground, MD 21010

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental Quality Branch
Preventive Medicine Division (HSHA-IPM)
Fort Sam Houston, TX 78234